

# Stabilization of organic carbon in chemically separated pools in no-till and meadow soils in Northern Appalachia

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## Abstract

Land use and soil management affects soil organic carbon (SOC) pools, chemical composition of stabilized SOC fractions, and the depth distribution. No-till production of corn (*Zea mays* L.) is a recommended management practice that reduces soil losses and increases SOC concentration, but the scientific knowledge of the mechanisms of C sequestration and protection is scanty. Therefore, the objective of this research was to compare the SOC pool, C pool in fine roots, and chemically separated C fractions with depth in three pedons from the same soil series: (i) meadow converted from no-till corn in 1988 (Meadow), (ii) continuous no-till corn since 1970 (NT); and (iii) continuous no-till corn with beef cattle manure since 1964 (NTm) at the North Appalachian Experimental Watershed near Coshocton, Ohio. The SOC pool ( $\text{Mg ha}^{-1}$ ) from 0–69 cm was the highest in NTm (76.2) and progressively smaller in NT (49.3) and Meadow (46.6) pedons. The SOC concentrations and pool sharply decreased with depth, but were always more in NTm than NT soil. Fine root C pool ( $\text{Mg ha}^{-1}$ ) was much larger in the pedon with perennial vegetation (Meadow, 1.28) than in those under corn (NT, 0.21; NTm, 0.09). The pool of chemically separated C fractions and their depth distribution varied depending on the separation technique. The amounts of C preferentially bound to soil minerals in 0–69 cm depth were comparable among pedons, as indicated by treatment with HF to release mineral-bound SOC. The NTm pedon had a larger pool of recalcitrant non-hydrolyzable C ( $58.4 \text{ Mg ha}^{-1}$ ), as indicated by HCl treatment. The Meadow pedon stored the smallest pool ( $3.4 \text{ Mg ha}^{-1}$ ) of oxidisable C, as was indicated by treatment with disodium peroxodisulfate ( $\text{Na}_2\text{S}_2\text{O}_8$ ). The relationship between chemically separated C fractions and turnover time of SOC at depth, however, warrants further studies. Nevertheless, the results indicate that no-till corn with added manure has a high potential for C sequestration by increasing the size of the SOC pool in the subsurface horizons.

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## 1. Introduction

Fossil fuel emissions and land-use changes have disturbed the natural balance between carbon (C) assimilation and respiration (Prentice et al., 2001). World soils are an essential part of the global C cycle. The soil organic C (SOC) pool is 3.3 times larger than the atmospheric pool and 4.5 times the biotic pool (Lal, 2004). To predict and ameliorate the consequences of climate and land cover change, a clear understanding of the distribution of SOC among diverse pools is required (Jobbágy and Jackson, 2000). Although many regional SOC budgets are available, the depth distribution of the SOC pool and accom-

panying relationships with climate and vegetation are poorly understood (Lorenz and Lal, 2005). The net gain of C in the soil is a function of the balance between inputs (i.e., net primary productivity plus any external inputs) and losses (i.e., decomposition, erosion, leaching) (Baldock et al., 2004).

For soils in Ohio, Tan et al. (2004b) reported that the SOC pool is largely concentrated in the top 30 cm depth, and is particularly sensitive to soil and crop management practices as well as other environmental and anthropogenic perturbations. Tan and colleagues observed that site variables such as soil taxonomic classification, texture, drainage, slope, and elevation greatly contributed to the variability in the surface SOC pool of croplands. These variables, however, explained only a small fraction of the variance in forestlands, implying that other factors may be more important to SOC sequestration. Organic C

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is not only added to the surface soil, but a significant portion of fresh C enters the SOC pool *in situ* from roots (Krull et al., 2005). Land use changes may, therefore, also affect the SOC pool in subsoil horizons by altering rooting patterns. Nevertheless, it is difficult to draw conclusions because of the small global database (Guo and Gifford, 2002). In general, conversion of forest to cropland leads to a loss of SOC pool, although this may not be the case for conversion to pastures (Murty et al., 2002).

Physical and chemical techniques can be used to determine the partitioning of SOC into different pools with variable turnover times and stability (Baldock et al., 2004). The passive or recalcitrant SOC can be estimated by determining non-hydrolyzable C in the residue remaining after hydrolysis with 6 M HCl, most commonly by incubation at 116 °C for 16 h (Paul et al., 2006). The non-specific nature of this approach, however, may produce biased results (Eusterhues et al., 2003). For example, acid hydrolysis preferentially removes fatty acids, and proteinaceous- and polysaccharide-type organic matter (OM) (Paul et al., 2006). Proteins and polysaccharides may originate from young as well as old and relatively stable C fractions (Knicker and Hatcher, 2001; Kiem et al., 2002). Recently deposited long-chain alkyls, waxes, lignin and other aromatics may resist acid hydrolysis (Paul et al., 2006). Therefore, Eusterhues et al. (2003) proposed two chemical techniques to identify and separate old and/or stable OM from labile C fractions. Compositional changes in SOC by oxidation with disodium peroxodisulfate ( $\text{Na}_2\text{S}_2\text{O}_8$ ) are similar to changes that occur as the result of humification, and the residue of the peroxodisulfate treatment is enriched with long-chain aliphatic compounds (Cuypers et al., 2002), which are a significant portion of chemically resistant SOC (Mikutta et al., 2005). Demineralization by hydrofluoric acid (HF) has also been proposed by Eusterhues et al. (2003). By this method, OM from the stabilized SOC pool, and preferentially mineral-bound OM is released. The released fraction contains fresh, soluble, probably microbial, biomass (i.e., proteins, amino acids) and carbohydrates (Schmidt and Gleixner, 2005). As the turnover time of the SOC pool increases with depth, the portion of stable C fractions separated by the chemical techniques may also increase (Gaudinski et al., 2000).

Previous research on three predominant soil series at the North Appalachian Experimental Watershed (NAEW), i.e., Berks (loamy-skeletal, mixed, active, mesic, Typic Dystrudepts), Coshocton (fine-loamy, mixed, active, mesic, Aquultic Hapludalfs), and Rayne (fine-loamy, mixed, active, mesic, Typic Hapludults), has indicated that the top 20 cm soil under meadow may have a large potential to sequester SOC through biochemical protection mechanisms (Tan et al., 2004a). No-till production of corn with or without the addition of manure is a recommended management practice in this region for increasing SOC concentrations in the upper 30 cm (Blanco-Canqui et al., 2005). Therefore, the objective of this study was to characterize the effects of no-till and meadow on vertical distribution of the pool of SOC and fine-root C, and chemically separated C fractions in soils at the NAEW using three different separation techniques. The hypothesis tested was that land use affects the depth distribution of C in various labile and stable SOC pool fractions.

## 2. Material and methods

### 2.1. Sites and soils

The SOC and fine-root C pools were studied in 3 pedons of Rayne silt loam at the NAEW near Coshocton, Ohio, USA. The NAEW was established in 1938, and is at 40°22' N and 81°48' W with an elevation of 300 to 600 m (Kelley et al., 1975). The mean annual precipitation is 950 mm and the mean annual temperature is 10.3 °C. The soils developed primarily from coarse-grain sandstone and shale bedrock with some interbedded limestone. The Rayne soil is dominated by silt (53 to 67%) in the upper part of the profile and sand (43 to 61%) in the subsoil, with clay content ranging from 15 to 26% throughout the profile (C. E. Redmond, Area Resource Soil Scientist, NRCS/USDA, pers. Communication). The three sampled pedons were each located in small watersheds. These were under the following land use and management practices: (i) orchardgrass meadow (*Dactylis glomerata* L.) used for hay production, converted from no-till corn in 1988 (Meadow), (ii) continuous no-till corn since 1970 (NT); and (iii) continuous no-till corn with beef cattle manure applied each spring at the rate of approx. 15 Mg ha<sup>-1</sup>, since 1964 (NTm). The no-till watersheds were amended each spring with 150 kg N ha<sup>-1</sup> as  $\text{NH}_4\text{NO}_3$  and weeds were controlled mainly by triazine and acetanilide herbicides.

### 2.2. Soil and fine root sampling

Soils and fine roots (<2 mm) were sampled from pits (2.2 m long, 1 m wide, and variable depth to bedrock) dug in each watershed at summit landscape positions located within 15 m distance during October/November 2004. Summit positions were selected to minimize differences among pedons due to erosion or deposition. After describing the profiles (Kelley et al., 1975), three disturbed soil samples from each horizon were taken to the depth of the bedrock by repeatedly using a soil probe (7.6 cm in diameter and 15.2 cm long). Mineral soil samples were obtained from the middle and both ends of the profile at the Meadow land use, and from three positions between corn rows for the NT and NTm watersheds after discarding loose crop residue and grass on surface of the pedons. Soil bulk density was measured using six undisturbed soil cores (5.4 cm in diameter and 6.0 cm deep) collected from each horizon (Grossman and Reinsch, 2002).

The cores were gently broken apart by hand, the fine roots were removed using tweezers after which the samples were passed through a 2-mm sieve. The coarse fragments (>2 mm) were dried at 105 °C and weighed. The soil was dried at 40 °C, and sub-samples for chemical treatments and analysis were ground using a mortar and pestle to pass through a 0.4-mm mesh sieve.

Fine roots separated from the soil cores were washed by ultrasonication in distilled water for up to 3 min, depending on the amount of biomass. After drying at 70 °C, the roots were allowed to settle for 2 min in distilled water, and dried again at 70 °C and immediately weighed. Fine root dry weight was corrected for soil contamination by combusting for 4 h at

550 °C and calculating the ash-free dry weight. For C determination, three sub-samples (pseudo-replicates) for each horizon were combined and ground with a disc mill.

### 2.3. Chemical separation

The samples were demineralized by adding 10 ml of 10% HF to 1 g of ground bulk soil (Schmidt et al., 1997). The suspension was hand-shaken for 30 s and allowed to react for 15 h. The supernatant was removed with a tube attached to a plastic syringe, and the HF treatment was repeated twice. The undissolved material was washed six times with distilled water and vacuum-filtered through 0.45- $\mu$ m pore-size cellulose-nitrate filter to remove HF. Recalcitrant SOM was determined by placing 1 g of ground bulk soil in a 200-ml digestion flask and adding 25 ml of 6 M HCl, and hand-shaking for 30 s (Paul et al., 2001). After incubation at room temperature for 16 h, the supernatant was removed by vacuum-filtration through a 0.45- $\mu$ m pore size cellulose-nitrate filter. The filter and residue were washed six times with distilled water. Oxidation of the SOC was achieved by treatment with Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (Meier and Menegatti, 1997). Briefly, 0.5 g of ground bulk soil was dispersed in 250 ml of distilled water and allowed to react with 20 g of Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, buffered with 22 g NaHCO<sub>3</sub> for 2 days at 80 °C. After oxidation, the samples were vacuum-filtered through a 0.45- $\mu$ m pore-size cellulose-nitrate filter and washed twice with distilled water. Finally, residues and filters from each treatment were dried for 3 days at 40 °C and immediately weighed. The isolated pellets were ground for chemical analysis using a mortar and pestle to pass through a 0.25-mm sieve.

### 2.4. Carbon and nitrogen determinations

Determination of C and N in the soil samples, the residues after demineralization, acid hydrolysis, wet oxidation, and the fine roots were done by using a CN analyzer (Vario Max CN Analyzer, Elementar GmbH, Hanau, Germany). Total C was assumed to be the SOC concentration as the inorganic C concentrations were negligible (<0.2%). The pool size (Mg ha<sup>-1</sup>) of C in bulk soil, fine roots and chemically separated fractions, and of N in bulk soil were calculated as the product of C and N concentrations, bulk density and depth of the specific soil layer, corrected for the contribution of the coarse fraction (>2 mm), respectively. To compare the pedons, depth-weighted C and N pools were calculated for 0–20 cm and 0–69 cm soil depths as the top 20 cm were plowed before establishing no-till, and the shallowest pedon (Meadow) was 69 cm deep.

### 2.5. Statistical analysis

The results of the soil chemical analyses are based on arithmetic means ( $\pm$ S.D.) of 3 samples from each soil horizon. For statistical validity of the results, all variables were tested for normality (Kolmogorov-Smirnov) and for homogeneity of variances (Levene's test). Data were log-transformed prior to analysis. Differences in means of soil and fine root C, soil N, HF-soluble SOC, and SOC not removed by HCl and by

Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub> for 3 samples from each horizon within each profile were tested by a one-way analysis of variance (ANOVA;  $P < 0.05$ ). This was also done by comparing data for the 3 sampling positions among soil profiles. Statistical analyses were done using Statistical Package for the Social Sciences (SPSS for Windows®, Ver. 10.1, Chicago, Illinois). Because watershed treatments are not replicated, sub-samples were treated as pseudo-replicates.

## 3. Results and discussion

### 3.1. Carbon and nitrogen in pedons and fine roots

The SOC concentrations within the soil profiles ranged from 1.5 to 24.1 g C kg<sup>-1</sup> (Table 1). Among all soil management and land use treatments, the highest SOC concentrations were observed in the uppermost horizon, where the input of C from plant litter was the highest (Jobbágy and Jackson, 2000). The SOC concentration decreased sharply from the uppermost to the underlying horizons due to decreasing input of surface litter C (Franzluebbers, 2002). The highest SOC concentration was measured in NTm, followed by Meadow and NT, although concentrations are not comparable due to varying thicknesses and depths of the horizons. The high SOC concentration at the NTm site is probably attributable to the application of cattle manure (Hao et al., 2003; Blanco-Canqui et al., 2005).

The C:N ratios in top horizons were comparable among pedons, and ratios decreased with depth due to progressive decomposition (Table 1; Batjes, 1996). The decline of C:N ratio with depth was, however, less pronounced in the NTm pedon. The C:N ratio is often the best predictor of decomposition rate and indicator for the extent of decomposition, but may also be misinterpreted as causal factor relative to gross biochemical composition and spatial arrangement of constituents in plant tissue (Nicolardot et al., 2001; Magid et al., 2004; Jensen et al., 2005).

The SOC pools in 0–20 cm and 0–69 cm depths were largest in the NTm pedon and were similar in the Meadow and NT pedons (Table 2). Likewise, the soil N pool was largest for both

Table 1  
Depth distribution of SOC, and C:N ratios in soil profiles

Land use	Horizon <sup>a</sup>	Depth (cm)	SOC (g C kg <sup>-1</sup> )	C:N ratio
Meadow	Ap	0–23	13.0 (1.9) a	11.1 (0.2) a
	B21	23–30	4.4 (0.6) b	10.2 (0.3) b
	B22	30–46	2.4 (0.1) c	8.8 (0.3) c
	B23	46–69	1.9 (0.4) c	7.9 (0.6) d
NT	Ap1	0–28	9.7 (3.3) a	11.1 (0.9) a
	Ap2	28–36	3.5 (0.3) b	9.1 (1.1) a
	B21t	36–51	2.0 (0.2) c	6.8 (0.3) b
	IIB22t	51–74	1.5 (0.6) c	6.2 (1.0) b
NTm	Ap	0–20	24.1 (4.4) a	10.8 (0.1) a
	B1	20–33	6.0 (1.2) b	9.7 (0.2) b
	B21t	33–56	3.1 (0.2) c	8.5 (0.3) c
	IIB22t	56–72	1.7 (0.2) d	8.2 (0.3) c

<sup>a</sup> Kelley et al. (1975).

(N=3 for SOC and C:N; S.D. in brackets; data not sharing a common letter are statistically different among horizons, ANOVA, Student–Newman–Keuls test,  $P < 0.05$ ).

Table 2  
Pool of soil C, N and fine root C for 0–20 cm and 0–69 cm depths

Land use	SOC	N	Fine root C	SOC	N	Fine root C
	(Mg ha <sup>-1</sup> )					
	0–20 cm			0–69 cm		
Meadow	29.9 (4.5) a	2.7 (0.4) a	0.85 (0.35) a	46.6 (4.8) a	4.5 (0.5) a	1.28 (0.47) a
NT	27.4 (8.5) a	2.4 (0.6) a	0.04 (0.01) b	49.3 (10.9) a	4.9 (0.8) a	0.21 (0.06) b
NTm	52.9 (9.9) b	4.9 (0.9) b	0.05 (0.04) b	76.2 (10.8) b	7.5 (1.0) b	0.09 (0.08) b

(N=3; S.D. in brackets; data not sharing a common letter are statistically different among land uses, ANOVA, Student–Newman–Keuls test,  $P<0.05$ ).

depth intervals for the NTm pedon, but did not differ among other pedons. Earthworm activity might have been accentuated in the NTm pedon by manure application and altered the depth distribution of SOC (Edwards et al., 1988). Besides enhancing decomposition, the OM from manure contributes to long-term C and N pools (Hao et al., 2003). The larger SOC pool in NTm, especially deeper in the subsoil, suggests a higher potential for C sequestration as the turnover time of SOC generally increases with depth (Gaudinski et al., 2000; Puget et al., 2005). SOC and N pools in the pedon under Meadow, which was converted from no-till corn in 1988, were probably reduced relative to the other treatments because some of the aboveground biomass was removed as hay (Puget et al., 2005).

The Meadow soil had significantly more C in fine roots in 0–20 cm and 0–69 cm depths than NT and NTm systems (Table 2). Temperate grasslands exhibit high root:shoot ratios indicating a high relative belowground C-allocation to fine roots in grassland sites (Jackson et al., 1996). The fine root-C pool in both pedons under corn was, however, distinctively smaller due to lower belowground C-allocation as indicated by lower root:shoot ratios (Allmaras et al., 2004). As corn harvest was already finished prior to fine root sampling, faster decomposition of corn roots compared to grass roots probably contributed to the smaller fine root-C pools in the NT and NTm pedons (Silver and Miya, 2001). Although the C pool in fine roots of NT was smaller than that in the Meadow site, the SOC pools were similar probably due to greater amounts of C returned to the soil surface in NT and

greater subsurface additions of C due to corn rhizodeposition and corn root turnover (Bolinder et al., 1999; Puget et al., 2005).

### 3.2. Chemically separated soil organic carbon fractions

Between 17.8% (NTm) and 32.4% (Meadow) of total SOC pool in the top mineral soil horizon was released upon dissolution of the mineral phase with HF (Table 3). This value increased up to 47.6% at a depth of 33–56 cm in the NTm site while, in contrast, no significant changes were observed with depth in the Meadow and NT pedons. High C losses in subsoil by HF were also reported by Dai and Johnson (1999) and Eusterhues et al. (2003). HF reacts with silica to form soluble fluoride complexes, but reacts also with Fe and Al. A large fraction of chemically or biologically labile SOC adsorbed and protected by the mineral matrix may be released by the HF treatment (Gélinas et al., 2001). Carbon may, however, also be released from free and occluded OM by this treatment. Thus, in the topsoil of the NTm pedon only a small proportion of total SOC pool was preferentially stabilized by minerals, whereas this proportion did not differ significantly among soil horizons in Meadow and NT pedons. Beside polysaccharides, proteins and amino acids are typically released by the HF treatment (Schmidt and Gleixner, 2005).

The concentrations (g kg<sup>-1</sup>) of HF-soluble SOC were the highest in the uppermost horizons of all 3 pedons, and sharply decreased with depth (Table 3). The HF-soluble SOC pools at

Table 3  
Carbon loss due to HF treatment, SOC resistant to HCl and to Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, and C balance of these fractions

Land use	Depth	HF-soluble SOC	SOC resistant to HCl	SOC resistant to Na <sub>2</sub> S <sub>2</sub> O <sub>8</sub>	HF-soluble SOC	SOC resistant to HCl	SOC resistant to Na <sub>2</sub> S <sub>2</sub> O <sub>8</sub>
	cm	% of total SOC			g kg <sup>-1</sup>		
Meadow	0–23	32.4 (2.4) a	79.2 (2.4) a	4.3 (0.7) a	4.2 (0.3) a	10.3 (1.8) a	0.6 (0.0) a
	23–30	38.2 (6.4) a	75.3 (0.9) a, b	10.1 (1.4) b	1.7 (0.5) b	3.3 (0.4) b	0.4 (0.0) b
	30–46	42.8 (5.4) a	70.8 (2.9) b	16.0 (2.0) c	1.0 (0.2) c	1.7 (0.1) c	0.4 (0.0) b
	46–69	34.8 (5.3) a	74.7 (2.5) a, b	20.9 (3.4) c	0.7 (0.2) c	1.4 (0.3) c	0.4 (0.0) b
NT	0–28	31.7 (2.7) a	73.4 (2.7) a	9.4 (0.5) a	3.0 (0.8) a	7.1 (2.2) a	0.9 (0.3) a
	28–36	35.1 (2.0) a	78.4 (1.2) a	17.7 (5.7) b	1.2 (0.2) b	2.8 (0.2) b	0.6 (0.2) a
	36–51	41.0 (6.5) a	78.8 (3.3) a	23.2 (5.4) b, c	0.8 (0.1) b, c	1.6 (0.2) b	0.5 (0.1) a
	51–74	30.6 (15.3) a	82.3 (8.1) a	37.3 (16.3) c	0.5 (0.3) c	1.2 (0.4) b	0.5 (0.0) a
NTm	0–20	17.8 (3.3) a	77.9 (1.6) a	4.9 (0.3) a	4.3 (1.3) a	18.7 (3.1) a	1.2 (0.1) a
	20–33	36.8 (5.1) b	76.7 (1.2) a	8.3 (1.4) b	2.2 (0.2) b	4.6 (0.9) b	0.5 (0.0) b
	33–56	47.6 (4.3) b	70.5 (2.3) a	13.9 (1.3) c	1.5 (0.2) c	2.2 (0.1) c	0.4 (0.0) b
	56–72	45.2 (3.1) b	75.9 (5.3) a	24.1 (3.0) d	0.8 (0.1) d	1.3 (0.1) c	0.4 (0.0) b

(N=3; S.D. in brackets; data not sharing a common letter within land uses are statistically different among horizons, ANOVA, Student–Newmans–Keuls test,  $P<0.05$ ).



Table 4  
Pool of C in chemically separated fractions for 0–20 cm and 0–69 cm depths

Land use	HF-soluble SOC (Mg ha <sup>-1</sup> )	SOC resistant to HCl	SOC resistant to Na <sub>2</sub> S <sub>2</sub> O <sub>8</sub>	HF-soluble SOC	SOC resistant to HCl	SOC resistant to Na <sub>2</sub> S <sub>2</sub> O <sub>8</sub>
	0–20 cm			0–69 cm		
Meadow	9.6 (0.8) a	23.7 (4.3) a	1.3 (0.1) a	15.8 (0.7) a	36.3 (4.7) a	3.4 (0.3) a
NT	8.5 (2.1) a	20.0 (5.7) a	2.6 (0.8) b	16.0 (2.4) a	36.7 (7.2) a	6.2 (1.1) b
NTm	9.5 (3.0) a	41.1 (6.8) b	2.6 (0.3) b	19.2 (2.2) a	58.4 (7.7) b	5.4 (0.5) b

(N=3; S.D. in brackets; data not sharing a common letter are statistically different among land uses, ANOVA, Student–Newman–Keuls test,  $P<0.05$ ).

0–20 cm and 0–69 cm were not significantly different among pedons (Table 4), but the SOC pool was larger at 0–69 cm depth in NTm than in the other treatments (Table 2). This indicates that NTm contained the largest amount of recalcitrant C among the three pedons, although fresh and soluble biomass may also have contributed to this pool (Eusterhues et al., 2003; Schmidt and Gleixner, 2005).

The C:N ratios of the residues remaining after the HF treatment generally decreased with depth (Table 5). The relative changes in C:N ratios after HF treatment were negative, indicating that C was preferentially lost relative to N (Schmidt and Gleixner, 2005). The changes in C:N ratios upon HF were smallest in the top horizons probably due to higher amounts of easily water- or acid-soluble OM than deeper in the profiles.

There is no standard method for acid hydrolysis (Tan et al., 2004a; Paul et al., 2006). Soil sugars may caramelize during 6 M HCl exposure at high temperatures (Paul et al., 2006). To reduce possible alterations of SOC, acid hydrolysis was performed at room temperature. This procedure probably results in extraction of mainly fulvic acids as incubation with 1 M HCl at room temperature is used to obtain a supernatant for isolation of fulvic acids (Swift, 1996). Thus, the regression slope between the amount of non-hydrolyzable C and the amount of total SOC was higher compared to hydrolyzing with hot HCl (0.77 vs. 0.51, not shown; Paul et al., 2006). As a result, the fractions of SOC resistant to HCl in uppermost horizons at Meadow and NT (Table 3) were distinctively greater than the 40.8% for Meadow and 38.3% for NT in 0–20 cm depth reported by Tan et al.

(2004a). More than 70% of total SOC pool resisted HCl treatment, and differences among horizons within each pedon were generally small. For all sites, the highest concentrations (g kg<sup>-1</sup>) of SOC resistant to HCl were observed in the uppermost mineral soil horizon, and concentrations strongly decreased with depth. The NTm pedon contained a larger pool of SOC resistant to HCl at the 0–20 cm and 0–69 cm depths compared to Meadow and NT pedons (Table 4). For a range of soils, SOC remaining after hydrolysis with hot 6 M HCl varied considerably from 30 to 80% of SOC depending on soil type, depth, texture, and management (Paul et al., 2006).

As observed with the HF-residue, C:N ratios of the HCl treatment residue decreased with depth in all pedons (Table 5). The relative changes in C:N ratios in the Meadow profile were variable, similar among horizons in NT, but generally became increasingly more negative with depth in NTm pedon. Negative relative changes in C:N ratios of HCl-resistant SOC indicated preferential C loss, probably as polysaccharide-type SOC fraction. Definitive interpretation of C:N ratios is problematic as mineral-bound NH<sub>4</sub><sup>+</sup> or NH<sub>4</sub><sup>+</sup> produced during SOC degradation also affects the ratios (Leifeld and Kögel-Knabner, 2001). The concentration of SOC resistant to HCl strongly decreased with depth (Table 3), while C:N ratios of the HCl-resistant residues narrowed (Table 5). Paul et al. (2001) explained this trend in depth distribution with missing lignin and leaf-wax-derived, long-chain aliphatics in root-derived substrates for humification. Within a given species, however, the average lignin content of roots is more than double of that in

Table 5  
C:N ratios of residues after HF treatment, HCl hydrolysis and oxidation with Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, and calculated alterations of C:N ratios

Land use	Depth (cm)	HF-insoluble SOC C:N	Relative change <sup>a</sup> (%)	SOC resistant to HCl C:N	Relative change (%)	SOC resistant to Na <sub>2</sub> S <sub>2</sub> O <sub>8</sub> C:N	Relative change (%)
Meadow	0–23	10.6 (0.2) a	–5	10.4 (0.2) a	–7	11.5 (1.4) a	+3
	23–30	7.6 (1.1) b, c	–25	7.8 (0.8) b	–23	7.4 (1.1) a	–27
	30–46	5.9 (0.5) d	–33	5.9 (0.5) c	–33	5.7 (0.5) a	–35
	46–69	6.9 (0.4) c, d	–14	5.7 (0.5) c	–28	6.0 (0.7) a	–24
NT	0–28	8.4 (1.0) a	–24	8.6 (0.7) a	–22	9.3 (4.2) a	–16
	28–36	5.8 (0.3) a, b	–36	7.0 (0.8) b	–23	5.2 (0.7) a	–43
	36–51	3.3 (0.7) b	–52	5.2 (0.4) c	–22	4.9 (0.8) a	–27
	51–74	3.8 (2.1) b	–38	4.8 (0.6) c	–23	6.6 (0.8) a	+7
NTm	0–20	6.0 (0.8) a	–45	9.8 (0.2) a	–9	16.5 (1.8) a	+53
	20–33	2.7 (0.7) b	–72	8.2 (0.3) b	–16	6.2 (0.4) b	–36
	33–56	2.8 (2.3) b	–67	6.2 (0.3) c	–27	5.2 (0.3) c	–39
	56–72	1.2 (0.2) b	–86	5.1 (0.4) d	–38	6.5 (0.6) b	–20

<sup>a</sup>Relative change (%) =  $100 \times (C:N_{\text{after}} - C:N_{\text{before}}) / (C:N_{\text{before}})$  (Schmidt and Gleixner, 2005).

(N=3; S.D. in brackets; data not sharing a common letter within land uses are statistically different among horizons, ANOVA, Student–Newman–Keuls test,  $P<0.05$ ).

shoots, and polyaliphatic suberin from root tissue is a major contributor to SOC (Bull et al., 2000; Nierop et al., 2003; Rasse et al., 2005). In summary, the NTm pedon had a larger pool of total and HCl-resistant SOC pool in the 0–69 cm depth interval than did the other pedons (Tables 2 and 4).

Between 4.3% (Meadow) and 9.4% (NT) of total SOC pool in the uppermost mineral soil horizon resisted treatment with  $\text{Na}_2\text{S}_2\text{O}_8$  (Table 3). In all profiles, this percentage strongly increased with depth reaching a maximum of 37.3% in the deepest horizon of the NT pedon, and 20.9% in the Meadow and 24.1% in the NTm pedons. A similar distribution was reported by Eusterhues et al. (2003) in a Spodosol and Cambisol under forest. The concentrations ( $\text{g kg}^{-1}$ ) and pools of C not removed by  $\text{Na}_2\text{S}_2\text{O}_8$  were, however, very small compared to the other chemical treatments (Tables 3 and 4). While the C concentrations resistant to  $\text{Na}_2\text{S}_2\text{O}_8$  decreased with depth in the Meadow and NTm pedons, no significant changes with depth occurred in the NT pedon (Table 3). The Meadow pedon had the smallest SOC pool resistant to  $\text{Na}_2\text{S}_2\text{O}_8$  at the 0–20 cm and 0–69 cm depths (Table 4). During peroxodisulfate treatment, OM is degraded by  $\text{SO}_4^-$  radicals and, to a lesser extent, by OH-radicals (Mikutta et al., 2005). The resistant, non-oxidisable fraction probably contains aromatic C structures, such as charcoal or black C (Schmidt et al., 1999). Furthermore, Cuypers et al. (2002) reported that SOC after peroxodisulfate treatment is preferentially enriched with long-chain aliphatics. The C removal efficiency during  $\text{Na}_2\text{S}_2\text{O}_8$  treatment depends, however, also on the protective capability of mineral surfaces (Mikutta et al., 2005). The percentages of HF-soluble SOC, indicative for the mineral-bound SOC, were comparable among pedons. The relatively lower resistance to  $\text{Na}_2\text{S}_2\text{O}_8$  in the Meadow may, therefore, be caused by a different chemical composition of SOC due to input of lignin and waxes from leaf and root litter. Root litter is generally high in lignin due to woody tissues while crop residues are high in polysaccharides (Lorenz and Lal, 2005). The SOC resistant to oxidation was present in smaller amounts than the HF-soluble SOC. Thus, only a small portion of the SOC stabilized by soil minerals was also resistant to chemical oxidation (Eusterhues et al., 2003). In summary, the Meadow pedon had the smallest pool of SOC not oxidisable by  $\text{Na}_2\text{S}_2\text{O}_8$ , and both corn pedons had higher, but comparable amounts. The proportion of stable C fractions separated by this method generally increased with depth indicating increasing turnover time of the SOC pool (Gaudinski et al., 2000).

The C:N ratios of the residue after  $\text{Na}_2\text{S}_2\text{O}_8$  treatment were comparable among horizons for Meadow and NT (Table 5). In contrast, the C:N ratio of the top horizon of NTm was significantly higher than those for the subsoil horizons. The relative changes in C:N ratios after oxidation were variable. Low C:N ratios indicated high stability of N compounds (Mikutta et al., 2005). Similar to the HCl treatment, however, the interpretation of C:N ratios after SOC removal is ill-defined.

#### 4. Conclusions

Land use and soil management significantly affected C and N pools, and their depth distribution. No-till corn with

manure contained the highest C and N pools. The addition of manure also increased the C and N pools in subsoil horizons compared to no-till corn without manure, which may be partially attributable to increased earthworm activity. The fine root C pool was the highest in the Meadow pedon, but the SOC pool was small relative to the other pedons, probably due to removal of aboveground biomass as hay. Thus in no-till soils, application of manure at an optimal rate has a high potential for C sequestration as it also increases SOC pool in subsoil horizons.

Land use and soil management significantly affected the pools of chemically separated C fractions and their depth distribution, but results varied depending on the separation technique used. The pool of chemically stabilized C was distinctively larger in the surface horizons than in the subsoil in all three pedons due to a large total SOC pool. The pedons did not differ in the pool of C associated with soil minerals and with free and occluded OM fractions. The NTm pedon, however, contained the largest pool of recalcitrant non-hydrolyzable C. Compared to no-till corn without manure, the pool of these fractions was also larger in NTm deeper in subsoil horizons. In contrast, the Meadow pedon contained the lowest amount of peroxodisulfate-labile C, indicating that different C fractions were distinguished by the chemical techniques. The relation between chemically separated C fractions and turnover time of SOC at depth, however, warrants further studies including analyses of mineralogy,  $^{14}\text{C}$  age and chemical composition (e.g., by carbon-13 solid-state cross-polarization magic angle spinning nuclear magnetic resonance spectroscopy ( $^{13}\text{C}$  CPMAS NMR)).

#### References

- Allmaras, R.R., Linden, D.R., Clapp, C.E., 2004. Corn-residue transformations into root and soil carbon as related to nitrogen, tillage, and stover management. *Soil Sci. Soc. Am. J.* 68, 1366–1375.
- Baldock, J.A., Masiello, C.A., Gélinais, Y., Hedges, J.I., 2004. Cycling and composition of organic matter in terrestrial and marine ecosystems. *Mar. Chem.* 92, 39–64.
- Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world. *Eur. J. Soil Sci.* 47, 151–163.
- Blanco-Canqui, H., Lal, R., Owens, L.B., Post, W.M., Izaurrealde, R.C., 2005. Strength properties and organic carbon of soils in the North Appalachian Region. *Soil Sci. Soc. Am. J.* 69, 663–673.
- Bolinder, M.A., Angers, D.A., Giroux, M., Laverdière, M.R., 1999. Estimating C inputs retained as soil organic matter from corn (*Zea mays* L.). *Plant Soil* 215, 85–91.
- Bull, I.D., Nott, C.J., Van Bergen, P.F., Poulton, P.R., Evershed, R.P., 2000. Organic geochemical studies of soils from the Rothamsted classical experiments — VI. The occurrence and source of organic acids in an experimental grassland soil. *Soil Biol. Biochem.* 32, 1367–1376.
- Cuypers, C., Grotenhuis, T., Nierop, K.G.J., Franco, E.M., de Jager, A., Rulkens, W., 2002. Amorphous and condensed organic matter domains: the effect of persulfate oxidation on the composition of soil/sediment organic matter. *Chemosphere* 48, 919–931.
- Dai, K.H., Johnson, C.E., 1999. Applicability of solid state  $^{13}\text{C}$ PMAS NMR analysis in Spodosols: chemical removal of magnetic materials. *Geoderma* 93, 289–310.
- Edwards, W.M., Shipitalo, M.J., Norton, L.D., 1988. Contribution of macroporosity to infiltration into a continuous corn no-tilled watershed: implications for contaminant movement. *J. Contam. Hydrol.* 3, 193–205.

- Eusterhues, K., Rumpel, C., Kleber, M., Kögel-Knabner, I., 2003. Stabilisation of soil organic matter by interactions with minerals as revealed by mineral dissolution and oxidative degradation. *Org. Geochem.* 34, 1591–1600.
- Franzluebbers, A.J., 2002. Soil organic matter stratification ratio as an indicator of soil quality. *Soil Tillage Res.* 66, 95–106.
- Gaudinski, J.B., Trumbore, S.E., Davidson, E.A., Zheng, S., 2000. Soil carbon cycling in a temperate forest: radiocarbon-based estimates of residence times, sequestration rates and partitioning of fluxes. *Biogeochemistry* 51, 33–69.
- Gélinas, Y., Baldock, J.A., Hedges, J.I., 2001. Demineralisation of marine and freshwater sediments for CP/MAS  $^{13}\text{C}$  NMR analysis. *Org. Geochem.* 32, 677–693.
- Grossman, R.B., Reinsch, T.G., 2002. Bulk density and linear extensibility. In: Dane, J.H., Topp, G.C. (Eds.), *Methods of Soil Analysis, Part 4. SSSA Soil Series*, vol. 5. SSSA, Madison, WI, pp. 201–225.
- Guo, L.B., Gifford, R.M., 2002. Soil carbon stocks and land use change: a meta analysis. *Glob. Chang. Biol.* 8, 345–360.
- Hao, X., Chang, C., Travis, G.R., Zhang, F.R., 2003. Soil carbon and nitrogen response to 25 annual cattle manure applications. *J. Plant Nutr. Soil Sci.* 166, 239–245.
- Jackson, R.B., Canadell, J., Ehleringer, J.R., Mooney, H.A., Sala, O.E., Schulze, E.-D., 1996. A global analysis of root distributions for terrestrial biomes. *Oecologia* 108, 389–411.
- Jensen, L.S., Salo, T., Palmason, F., Breland, T.A., Henriksen, T.M., Stenberg, B., Pedersen, A., Lundström, C., Esala, M., 2005. Influence of biochemical quality on C and N mineralisation from a broad variety of plant materials in soil. *Plant Soil* 273, 307–326.
- Jobbágy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecol. Appl.* 10, 423–436.
- Kelley, G.E., Edwards, W.M., Harrold, L.L., McGuinness, J.L., 1975. *Soils of the North Appalachian Experimental Watershed. Miscellaneous Publication*, vol. 1296. ARS and SCS-USDA, Washington D.C.
- Kiem, R., Knicker, H., Kögel-Knabner, I., 2002. Refractory organic carbon in particle-size fractions of arable soils I: distribution of refractory carbon between the size fractions. *Org. Geochem.* 33, 1683–1697.
- Knicker, H., Hatcher, P., 2001. Sequestration of organic nitrogen in the sapropel from Mangrove Lake, Bermuda. *Org. Geochem.* 32, 733–744.
- Krull, E.S., Skjemstad, J.O., Burrows, W.H., Bray, S.G., Wynn, J.G., Bol, R., Spouncer, L., Harms, B., 2005. Recent vegetation changes in central Queensland, Australia: evidence from  $\delta^{13}\text{C}$  and  $^{14}\text{C}$  analyses of soil organic matter. *Geoderma* 126, 241–259.
- Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food security. *Science* 304, 1623–1627.
- Leifeld, J., Kögel-Knabner, I., 2001. Organic carbon and nitrogen in fine soil fractions after treatment with hydrogen peroxide. *Soil Biol. Biochem.* 33, 2155–2158.
- Lorenz, K., Lal, R., 2005. The depth distribution of soil organic carbon in relation to land use and management and the potential of carbon sequestration in subsoil horizons. *Adv. Agron.* 88, 35–66.
- Magid, J., Luxhøi, J., Lyshede, O.B., 2004. Decomposition of plant residues at low temperatures separates turnover of nitrogen and energy rich tissue components in time. *Plant Soil* 258, 351–365.
- Meier, L.P., Menegatti, A.P., 1997. A new, efficient, one-step method for the removal of organic matter from clay-containing sediments. *Clay Miner.* 32, 557–563.
- Mikutta, R., Kleber, M., Kaiser, K., Jahn, R., 2005. Review: organic matter removal from soils using hydrogen peroxide, sodium hypochlorite, and disodium peroxodisulfate. *Soil Sci. Soc. Am. J.* 69, 120–135.
- Murty, D., Kirschbaum, M.U.F., McMurtrie, R.E., McGilvray, H., 2002. Does conversion of forest to agricultural land change soil carbon and nitrogen? A review of the literature. *Glob. Chang. Biol.* 8, 105–123.
- Nicolardot, B., Recous, S., Mary, B., 2001. Simulation of C and N mineralization during crop residue decomposition: a simple dynamic model based on the C:N ratio of the residues. *Plant Soil* 228, 83–103.
- Nierop, G.J.K., Naafs, D.F.W., Verstraten, J.M., 2003. Occurrence and distribution of ester-bound lipids in Dutch coastal dune soils along a pH gradient. *Org. Geochem.* 34, 719–729.
- Paul, E.A., Collins, H.P., Leavitt, S.W., 2001. Dynamics of resistant soil carbon of midwestern agricultural soils measured by naturally occurring  $^{14}\text{C}$  abundance. *Geoderma* 104, 239–256.
- Paul, E.A., Morris, S.J., Conant, R.T., Plante, A.F., 2006. Does the acid hydrolysis–incubation method measure meaningful soil organic carbon pools? *Soil Sci. Soc. Am. J.* 70, 1023–1035.
- Prentice, I.C., Farquhar, G.D., Hibbard, K.A., et al., 2001. The carbon cycle and atmospheric carbon dioxide. *Climate change 2001: The Scientific Basis. Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge*, pp. 183–237.
- Puget, P., Lal, R., Izaurralde, C., Post, M., Owens, L., 2005. Stock and distribution of total and corn-derived soil organic carbon in aggregate and primary particle fractions for different land use and soil management practices. *Soil Sci.* 170, 256–279.
- Rasse, D.P., Rumpel, C., Dignac, M.-F., 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilization. *Plant Soil* 269, 341–356.
- Schmidt, M.W.I., Gleixner, G., 2005. Carbon and nitrogen isotope composition of bulk soils, particle-size fractions and organic material after treatment with hydrofluoric acid. *Eur. J. Soil Sci.* 56, 407–416.
- Schmidt, M.W.I., Knicker, H., Hatcher, P.G., Kögel-Knabner, I., 1997. Improvement of  $^{13}\text{C}$  CPMAS NMR spectra of bulk soils, particle size fractions and organic material by treatment with 10% hydrofluoric acid. *Eur. J. Soil Sci.* 48, 319–328.
- Schmidt, M.W.I., Rumpel, C., Kögel-Knabner, I., 1999. Particle size fractionation of soil containing coal and combusted particles. *Eur. J. Soil Sci.* 50, 515–522.
- Silver, W.L., Miya, R.K., 2001. Global patterns in root decomposition: comparisons of climate and litter quality effects. *Oecologia* 129, 407–419.
- Swift, R.S., 1996. Organic matter characterization. In: Sparks, D.L. (Ed.), *Methods of Soil Analysis, Part 3, SSSA Soil Series No. 5. SSSA, Madison, WI*, pp. 1011–1069.
- Tan, Z.X., Lal, R., Izaurralde, R.C., Post, W.M., 2004a. Biochemically protected soil organic carbon at the North Appalachian Experimental Watershed. *Soil Sci.* 169, 423–433.
- Tan, Z.X., Lal, R., Smeck, N.E., Calhoun, F.G., 2004b. Relationships between surface soil organic pool and site variables. *Geoderma* 121, 187–195.